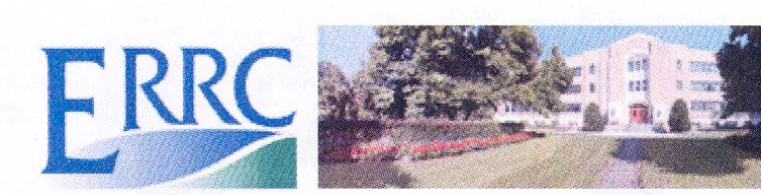

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Title: Inactivation of *Escherichia coli* in Apple Juice by Radio Frequency Electric Fields

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Inactivation of *Escherichia coli* in Apple Juice by Radio Frequency Electric Fields

D.J. GEVEKE AND C. BRUNKHORST

ABSTRACT: Heat pasteurization may detrimentally affect the quality of fruit and vegetable juices; hence, nonthermal pasteurization methods are actively being developed. Radio frequency electric fields processing has recently been shown to inactivate yeast in water at near-ambient temperatures. The objective of this study was to extend the radio frequency electric fields (RFEF) technique to inactivate bacteria in apple juice. A converged-field treatment chamber was developed that enabled high-intensity RFEF to be applied to apple juice using a 4-kW power supply. Finite element analyses indicated that uniform fields were generated in the treatment chamber. *Escherichia coli* K12 in apple juice was exposed for 0.17 ms to electric field strengths of up to 26 kV/cm peak over a frequency range of 15 to 70 kHz. The population of *E. coli* was reduced by 1.8 log following exposure to an 18 kV/cm field at an outlet temperature of 50 °C. Raising the temperature increased inactivation. Intensifying the electric field up to 16 kV/cm increased inactivation; however, above this intensity, inactivation remained constant. Radio frequencies of 15 and 20 kHz inactivated *E. coli* better than frequencies of 30 to 70 kHz. Inactivation was independent of the initial microbial concentration between 4.3 and 6.2 log colony-forming units (CFU)/mL. Applying 3 treatment stages at 50 °C increased inactivation to 3 log. The electric energy for the RFEF process was 300 J/mL. The results of the present study provide the 1st evidence that RFEF processing inactivates bacteria in fruit juice at moderately low temperatures.

Keywords: radio frequency, high electric fields, nonthermal, pasteurization, treatment chamber

Introduction

Nearly all fruit juice is pasteurized to ensure its safety. Pasteurization typically involves heating the juice to a high temperature and holding for a sufficient length of time. Although this certainly is effective at inactivating pathogenic microorganisms, it also alters the properties of the juice. Extensive research has been conducted on nonthermal processes that inactivate microorganisms without damaging the original attributes of the juice. For more than 15 y, high electric fields in liquid foods have been studied (Dunn and Pearlman 1987). Pulsed electric fields (PEF) at 35 kV/cm were applied to orange juice for 59 μs at 60 °C, and the quality of the juice was compared with that of juice pasteurized with hot water at 95 °C for 30 s (Yeom 2000). The PEF treatment prevented the growth of microorganisms at 37 °C for 112 d, and the PEF-treated juice retained more of the vitamin C and flavor compounds than the heat-treated juice.

Nonetheless, the PEF process has yet to be commercialized. The reasons for this are mainly economic. The energy requirement for complete pasteurization using PEF is estimated at 100 to 400 J/mL (Schoenbach 2002). By comparison, thermal pasteurization with heat regeneration requires as little as 30 to 40 J/mL. Pulsed electric fields equipment is extremely specialized. The high cost of the pulse generator is a problem confronting the industrial application of PEF processing (Jeyamkondan 1999). At a high pulse frequency and large scale of operation for industrial applications, the charging power supply and high-speed electrical switch are the major costs of the pulse generator (Zhang 1995a).

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Radio frequency electric fields (RFEF) processing is similar to PEF processing in that high electric fields are applied to liquids for extremely short durations at moderately low temperatures to inactivate microorganisms by electroporation (Zimmermann 1986). When an external electric field is applied to a cell in a suspension, an induced voltage is formed across the membrane owing to the membrane's capacitance. As the voltage is increased, the opposite charges on either side of the membrane are attracted to each other with greater force, and the membrane becomes thinner. At a sufficiently high voltage, pores are formed in the membrane and the cell ruptures. Whereas a PEF generator consists of a charging power supply and high-speed electrical switch, a RFEF generator consists of a simple AC power supply. Geveke and others applied a RFEF of 0.5 kV/cm at a frequency of 18 MHz to *Escherichia coli* K-12, *Listeria innocua*, and yeast in apple cider, beer, deionized water, and tomato juice and concluded that there were no nonthermal effects (Geveke 2002b). On the basis of their findings, Geveke and others designed and assembled a RFEF experimental system that applied much higher electric field strengths of 30 kV/cm at 20 kHz to suspensions of *Saccharomyces cerevisiae* in water (Geveke 2002a). The population of *S. cerevisiae* was reduced by more than 2 log at 40 °C, increasing the field strength, number of treatment stages, and temperature-enhanced inactivation. Varying the frequency between 20 and 60 kHz had no effect on inactivation (Geveke and Brunkhorst 2003). Radio frequency electric fields processing inactivated *E. coli* in saline water by 5 log at 74 °C in less than 1 s (Uemura and Isobe 2002). *Bacillus subtilis* spores in orange juice were reduced by 4 log using RFEF processing at 121 °C under pressurized conditions to elevate the boiling point (Uemura and Isobe 2003).

The objective of this work was to extend the RFEF process to treating apple juice containing bacteria at moderately low temperatures.

Materials and Methods

Microorganisms

P.M. Fratamico, a lead scientist at the U.S. Dept. of Agriculture, Wyndmoor, Pa., U.S.A., supplied the *E. coli* K12 substrain C600 (Fratamico 1993). The bacteria were maintained on tryptose agar (Difco Laboratories, Detroit, Mich., U.S.A.) at 4 °C. The *E. coli* K12 was cultured in brain heart infusion (Difco Laboratories) for 24 h at 37 °C. Apple juice (Brix approximately 12; viscosity approximately 1 cp) was purchased from a local store. A sample was analyzed for microorganisms and none were detected. The juice was inoculated from the stationary phase culture to give an approximately 4, 5, or 6 log colony-forming units (CFU)/mL population, depending on the experiment. The solution's pH was 4.0 and its conductivity was 2.1 mS/cm.

Equipment

Geveke and Brunkhorst have previously described the RFEF power supply system used in this investigation (Geveke and Brunkhorst 2003). The RFEF power supply produced a peak voltage of 5.2 kV over a frequency range of 15 to 70 kHz. It consisted of four 1-kW RF amplifiers (model 1000A; Industrial Test Products, Port Washington, N.Y., U.S.A.) and 4 step-up transformers (Industrial Test Products). These were connected in series. A function generator (model AFG 310; Tektronix, Beaverton, Ore., U.S.A.) drove the amplifiers.

The RFEF power supply and treatment chamber combination previously used in the study of yeast inactivation in water was incapable of applying high electric fields to apple juice. The juice's higher conductivity required more power than the RFEF power supply could generate. Therefore, a smaller and more uniform treatment chamber was designed and fabricated that could be used with the existing power supply to deliver high electric fields to apple juice. The chamber was made of Delrin, an acetal homopolymer (McMaster-Carr Supply, New Brunswick, N.J., U.S.A.). Delrin is a good electrical insulator, can be machined with standard tooling, and has good chemical resistance. The treatment chamber was designed to converge the apple juice into a narrow flow area to reduce the power requirement (Matsumoto 1991; Sensoy 1995). Juice entered and exited the Delrin chamber through the annuli of cylindrical stainless-steel electrodes (part nr SS-400-1-OR Swagelok; Solon, Ohio, U.S.A.), as shown in Figure 1. The output of the RFEF power supply was connected to the electrodes. The central part of the chamber consisted of a cylindrical gap having a 0.1-cm dia and a 0.2-cm length. Thus, the maximum electric field strength used in the study was 26 kV/cm obtained by dividing the peak voltage, 5.2 kV, by the length of the gap, 0.2 cm. A 0.3-cm space between the end of each of the electrodes and the central gap prevented arcing.

QuickField™ (Tera Analysis Ltd, Svendborg, Denmark) finite element analysis software was used to model the anisotropic AC current flow within the treatment chamber. Figure 2 presents the model's results for an electric field strength of 18 kV/cm. The apple juice flows through the electrode and enters a field-free region. It then flows into the cylindrical gap where the field is quickly raised to 18 kV/cm. The field within the gap is nearly uniform, which ensures that all of the juice is treated equally. The uniformity improves the energy efficiency of the process. By minimizing the regions within the treatment chamber where the electric field is too low to inactivate bacteria and only heats the juice, approximately < 5 kV/cm, the energy loss is minimized. Similarly, by minimizing the regions where the field is higher than needed to inactivate bacteria, the energy loss is minimized. Thus, the outlet temperature is lessened, and the apple juice is not over-treated.

The input voltage to the amplifiers and the supplied voltage and current to the RF treatment chamber were measured using an oscilloscope (model TDS210; Tektronix), current probe (model 411; Pearson Electronics, Palo Alto, Calif., U.S.A.), and a voltage divider (model VD15-8.3-A-KB-A; Ross Engineering, Campbell, Calif., U.S.A.).

The experimental system included a stainless-steel feed tank and a peristaltic pump (driver model 7523-40; head model 77200-62; Cole-Parmer, Vernon Hills, Ill., U.S.A.) that supplied the apple juice to the RFEF system at a flow rate of 550 mL/min through Norprene pump tubing (model 06402-15; Cole-Parmer). Turbulent flow within the treatment chamber (Reynolds Number = 12000) further improved the processing uniformity. The juice was exposed to intense RFEF for approximately 170 μs. At a frequency of 30 kHz, approximately 5 cycles would occur in 170 μs. The inlet temperature to the RF treatment chamber was controlled using a stainless-steel heat exchanger (model SC0004; Madden Manufacturing, Elkhart, Ind., U.S.A.) and a temperature controller (model CALL 9400; Cole-Parmer). The outlet temperature from the RF treatment chamber was 45 °C, 50 °C, or 55 °C, depending on the experiment.

The temperatures of the apple juice immediately before and after the RFEF treatment chamber were measured with fiber-optic sensors (model 790; Luxtron, Santa Clara, Calif., U.S.A.). The temperatures were continuously logged to a data acquisition system (Dasylab version 5.0; Dasytec USA, Amherst, N.H., U.S.A.).

The apple juice was quickly cooled after exiting the treatment chamber to < 25 °C using a stainless-steel cooling coil submerged

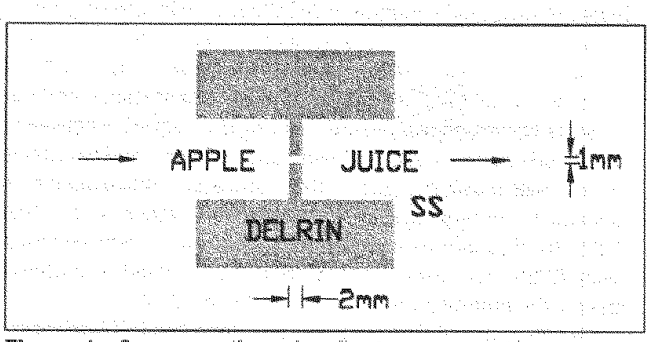

Figure 1—Cross-section of radio frequency electric fields (RFEF) converged treatment chamber including Delrin insulation and stainless-steel electrodes

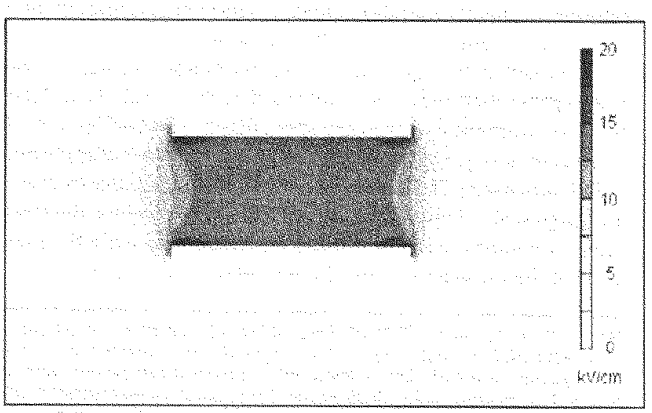

Figure 2—Modeled anisotropic AC current flow within the treatment chamber

in a water bath. The length of time for the juice to travel from the treatment chamber to the cooling coil was approximately 4 s.

In some cases, the effect of exposure to multiple treatment stages was desired, and the apple juice was reprocessed once or twice more, depending on the experiment. Product from the outlet of the cooler was collected in a carboy and was processed through the system a 2nd or 3rd time.

Controls were performed to determine the effect of temperature alone. The apple juice was heated to the desired temperature using the heat exchanger and then cooled using the cooling coil. The length of time for the juice to travel from the heat exchanger to the cooling coil was approximately 8 s.

Each experiment was performed in duplicate. Results were expressed as the means of these values ± standard deviations. The significance of differences in the RFEF results, based on the critical value of the Student *t* test, was calculated using Microsoft Excel statistical analysis algorithms.

Sampling and analysis

Duplicate samples were taken of the products. Appropriate dilutions of the samples were plated on tryptose agar using a spiral plater (model Autoplate 4000; Spiral Biotech, Bethesda, Md., U.S.A.) and incubated at 37 °C for 24 h. Enumerations were made with a colony counter (model CASBA 4; Spiral Biotech).

Results and Discussion

Radio frequency electric fields successfully inactivated *E. coli* K12 in apple juice at nonthermal conditions. The extent of microbial inactivation is dependent on the electric field strength (up to 16 kV/cm), number of treatment stages, frequency, and temperature.

A series of experiments were performed at 20 kHz to determine the effects of electric field strength and temperature on inactivation, and the results are presented in Figure 3. The population of *E. coli* was reduced by 1.4 ± 0.1 log after being exposed to a 24 kV/cm peak electric field at a treatment chamber inlet temperature of 10 °C and outlet temperature of 45 °C. The temperature increase in the RFEF treatment chamber was because of ohmic heating. When the electric field was eliminated and the inlet temperature was raised to match the outlet temperature, 45 °C, the reduction was <0.1 log, as shown in Figure 4. Applying an electric field of 24 kV/cm at an outlet temperature of 50 °C reduced *E. coli* by 1.9 ± 0.1 log. The vast majority of this reduction was because of nonthermal effects considering that the control, which had a longer come-up and hold time, was only 0.2 ± 0.1 log. The nonthermal inactivation is believed to be because of electroporation of the cells as a result of high electric fields (Zimmermann 1974). Geveke and Brunkhorst applied RFEF to *S. cerevisiae* in water, albeit with a different treatment chamber, and obtained an inactivation of 2.1 ± 0.1 log at 30 kV/cm and 40 °C (Geveke and Brunkhorst 2003). The results of the present study, with a newly designed treatment chamber, extend the RFEF process to the inactivation of bacteria in fruit juice.

Inactivation increased significantly as the electric field strength increased up to 16 kV/cm (critical value of Student *t* test, $P < 0.05$). However, inactivation remained constant with field strength above 16 kV/cm ($P > 0.1$), especially at 45 °C and 50 °C. Jayaram and others (1992) applied PEF to *Lactobacillus brevis* and observed similar behavior. Inactivation of *L. brevis* greatly increased with field strength up to 15 kV/cm, whereas, at higher fields, inactivation remained constant at temperatures between 30 and 45 °C. Wouters and others (1999) reported similar results for PEF treatment of *Listeria innocua* except that the threshold field strength was higher, 30 kV/cm, and was observed between 45 °C and 60 °C.

Experiments were conducted over the frequency range of 15 to 70 kHz, as shown in Figure 5. A 20 kV/cm electric field strength at a temperature of 50 °C was applied to *E. coli*. Significantly greater inactivation occurred at frequencies less than or equal to 20 kHz ($P < 0.01$). The cause of this has yet to be determined. Geveke and Brunkhorst (Geveke and Brunkhorst 2003) applied RFEF to *S. cerevisiae* in water at frequencies of 20, 40, and 60 kHz and concluded that frequency had no effect on inactivation. The variation in results may be because of the use of different microorganisms, media, or treatment chambers.

The effect of initial concentration on inactivation was studied. A 17 kV/cm electric field strength at a temperature of 45 °C was applied to *E. coli* having initial concentrations of 4.3, 5.4, and 6.2 log CFU/mL. The inactivations varied from 1.0 to 1.1 log and were not significantly different across the range of initial concentrations studied ($P > 0.1$). These results are in agreement with those of Zhang and others for PEF treatment of *E. coli* in ultra-filtrated sim-

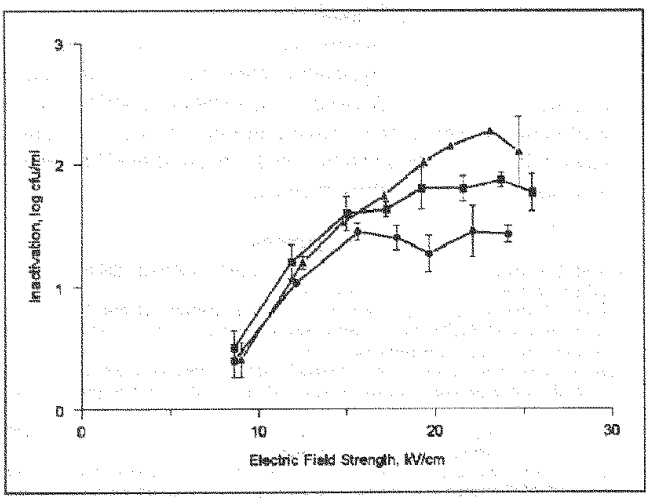

Figure 3—Effects of temperature and electric field strength on the inactivation of *Escherichia coli* at 20 kHz and with a 4-s hold time. ● = 45 °C; ■ = 50 °C; ▲ = 55 °C. Error bars indicate standard deviations.

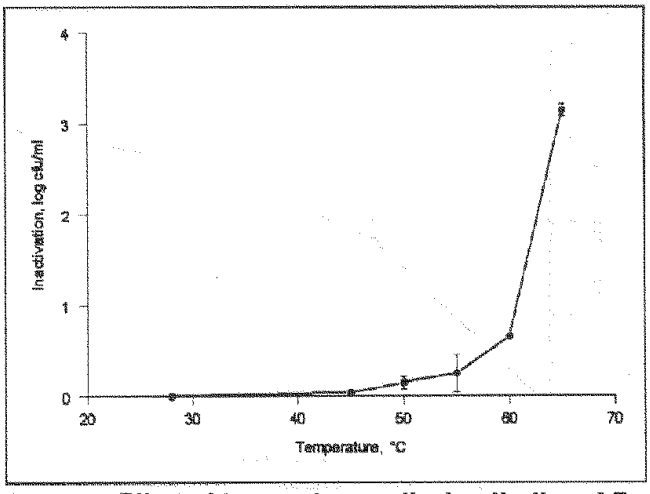

Figure 4—Effect of temperature on the inactivation of *Escherichia coli* with an 8-s hold time. Error bars indicate standard deviations.

ulated milk. Initial concentration, which ranged from 3 to 8 log CFU/mL, had no effect on inactivation (Zhang 1995b). However, earlier Zhang and others found that PEF inactivation of *S. cerevisiae* in apple juice was inversely affected by the initial concentration over the span of 4 to 6 log CFU/mL (Zhang 1994). These results were attributed to a cluster protection mechanism. Recently, Damar and others inactivated *E. coli* in peptone solution at initial concentrations between 3 to 8 log CFU/mL with PEF (Damar 2002). Inactivation was found to be inversely proportional to initial concentration and was presented as further support of a cluster protection mechanism. Once again, the discrepancy in results may be because of differences in the process parameters, microorganisms, or media used.

Inactivation increased with increasing number of treatments stages ($P < 0.05$) as shown in Figure 6. The juice was exposed to RFEF for approximately 170 μs during each stage. A single treatment of an 18 kV/cm electric field at 50 °C reduced the population of *E. coli* by 1.8 ± 0.3 log, whereas 3 stages of treatment resulted in a 3.0 ± 0.5 log reduction. Inactivation of recycled material was less than that of untreated material. This may be because of the more RFEF-sensitive *E. coli* having already been eliminated in the 1st treatment stage. Based on the flow rate, 550 mL/min, and the voltage and current measured by the oscilloscope, 2.5 kV_{rms} and 0.36 A_{rms}, respectively, the energy applied during each stage was 100 J/mL. From the inlet temperature, 30 °C, the energy calculated to raise the temperature of the apple juice to 50 °C is 88 J/mL. This is in good agreement with the energy calculated using the current and voltage. For 3 treatment stages, the total energy was 300 J/mL. The estimated energy required for pasteurization using PEF ranges from 100 to 400 J/mL (Barsotti and Cheftel 1999; Schoenbach 2002). Based on the U.S. Dept. of Energy's data for the average industrial electric price for 2002 of $0.047/kWh, the energy cost for the RFEF process was $0.015/gallon of apple juice. For comparison, conventional thermal pasteurization, with heat regeneration or recovery, requires only $0.002/gallon.

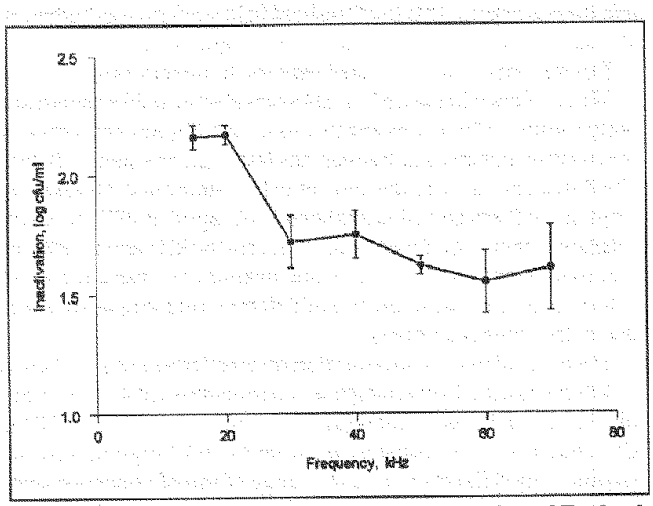

Figure 5—Effect of frequency on the inactivation of *Escherichia coli* at 20 kV/cm and with a 4-s hold time at 50 °C. Error bars indicate standard deviations.

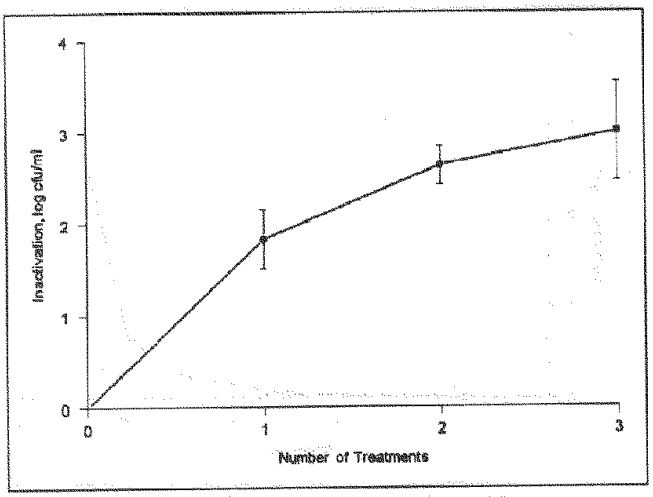

Figure 6—Effect of number of treatment stages on the inactivation of *Escherichia coli* at 50 °C, 20 kHz, and 18 kV/cm with a 4-s hold time. Error bars indicate standard deviations.

Additional studies are recommended. The RFEF process needs to be scaled up to be of commercial interest. In addition, greater inactivations than 3 log are necessary. To accomplish this, an 80-kW RF power supply has been purchased and a new RFEF system is being assembled. The additional power should enable the processing of greater flow rates. In addition, the new system should be able to provide power to several treatment chambers in series, thus eliminating the need for recycling.

Conclusions

Radio frequency electric fields significantly reduced the population of *E. coli* K12 in apple juice at 45 °C. This is the 1st well documented evidence that RFEF inactivate bacteria in juice at moderately low temperatures. Inactivation is dependent upon the temperature, number of treatment stages, and electric field strength up to 16 kV/cm. Above this level, inactivation is independent of field strength. Significantly greater inactivation occurs at radio frequencies of 15 and 20 kHz as compared with frequencies of 30 to 70 kHz. Inactivation is independent of initial microbial concentration in the range of 4 to 6 log CFU/mL. A 3 log reduction can be obtained in a 3-step process at 18 kV/cm and 50 °C. The calculated electrical cost is $0.015/gallon of apple juice. The RFEF process should be capable of pasteurizing vegetable and fruit juices at moderately low temperatures by increasing the number of treatment stages.

Acknowledgments

Mention of brand or firm name does not constitute an endorsement by the U.S. Dept. of Agriculture over others of similar nature not mentioned. We thank A. Bigley for assistance in performing the experiments, as well as O. J. Scullen for microbiological support. Princeton Plasma Physics Laboratory is funded by the U.S. Dept. of Energy and managed by Princeton Univ.

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